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A Novel and De Novo Spontaneous Point Mutation (Glu271STOP) of the Antithrombin Gene Results in a Type I Deficiency and Thrombophilia

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We describe a novel, de novo point mutation in one antithrombin (AT) allele resulting in type I AT deficiency and thrombophilia. Low plasma AT activity as well as low plasma AT antigen were documented in the propositus, but not in the parents, or in a male sibling. AT gene analysis by sequencing polymerase chain reaction-amplified genomic DNA from exon 5 of the propositus revealed a novel point mutation, GAG→TAG at codon 271, resulting in a stop codon (Glu271STOP). This mutation was not demonstrable in the other members of his immediate family. DNA marker polymorphism analysis indicated the expected parentage. Based on allele frequency data for Caucasians in the United States the cumulative paternity index, or CPI, for the propositus and his father is 219,077. This corresponds to a probability of paternity of 99.9995% based on a prior probability of 50%. Included in this analysis is a linkage analysis of a trinucleotide repeat in intron 5 of the AT gene of the various family members, which also confirmed maternity and paternity. These studies provide documentation of the first spontaneous mutation of an AT gene in a thrombophilic individual, resulting in a type I AT deficiency. Am. J. Hematol 60:126–129, 1999. © 1999 Wiley-Liss, Inc.

Key words: antithrombin; antithrombin deficiency; thrombophilia; type I AT deficiency

INTRODUCTION

Antithrombin (AT) is the major circulating plasma inhibitor of thrombin and the other serine proteases of coagulation. The gene for AT, on chromosome 1q 23–25, spans over 13 kilobase pairs, consists of seven exons, and encodes a mature polypeptide of 432 amino acids. Deficiency of AT has been described clinically and phenotypically by either reduced antigenic and functional levels (type I), or by reduced function but normal antigenic levels (type II). Genotypically, AT deficiency has been defined by autosomally inherited mutations in an AT allele ranging from a point mutation to a whole gene deletion [1–4].

Of the mutations recorded in the AT mutation database, those causing type II AT deficiency outnumber those causing type I deficiency [1–4]. The most recent AT mutation database update reported a substantial increase in the number of type I AT mutations, however [1]. These consist of frameshift deletions and insertions, missense and nonsense mutations, as well as partial and whole gene deletions. Of the documented mutations causing type II AT deficiency, most result in AT molecules with heparin binding dysfunction, followed, in order of decreasing frequency, by reactive site and pleiotropic defects. Missense mutations are the most common reported type of genetic defect. A similar number of nonsense, deletion and insertion mutations have been reported. Of the AT mutations causing type I AT deficiency reported, affected siblings or other family members have always been identified. We describe the first

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TABLE I. Diagnostic Laboratory Test Results for the Propositus*

Test	Result (normal range)[a]
AT antigen	17 mg/dl (22–39 mg/dl)
AT activity	54% (80–150%)
HCII	131% (65–140%)
Antiphospholipid antibody	Negative
PC activity	77% (65–175%)
PS (free)	119% (65–150%)
APCR screening test	2.5 (2.0–4.0)
Plasminogen	91% (75–130%)

*AT, antithrombin; HCII, heparin cofactor II; PC, protein C; PS, protein S; APCR, activated protein C resistance.
[a]Values in parentheses are the clinical laboratory normal reference ranges.

TABLE II. Genetic Marker Studies Performed in This Kindred to Confirm Paternity and Maternity

Genetic marker	Chromosome location	# alleles	Reference
AT3-STR	1q23	12	5
HLA-DQ1a	6p21.3	7	6
LDLR	19p13.1-13.3	2	6
GYPA	4q28-31	2	6
HBGG	11p15.5	3	6
D7S8	7q22-31.1	2	6
GC	4q11-13	3	6
HUMTPOX	2p23-pter	5	7
HUMCSF1PO	5q33-34	7	7
HUMTH01	11p11.5	7	7

TABLE III. Genetic Marker Study Results*

Genetic marker	Mother	Father	Propositus	Sibling	Paternity index of propositus
AT3-STR	14/14	16/17	16/14	17/14	4.27
CSF1PO	10/11	9/11	10/9	10/9	15.15
TPOX	11/11	8/11	11/11	11/11	1.75
TH01	6/7	7/7	7/7	7/7	7.09
LDLR	A/A	A/A	A/A	A/A	2.23
GYPA	A/A	A/A	A/A	A/A	1.89
HBGG	A/A	B/B	A/B	A/B	2.22
D7S8	B/B	A/A	B/A	B/A	1.64
GC	A/C	A/B	A/A	A/C	2.81
DQA1	2/3	1.1/1.1	3/1.1	2/1.1	6.33

*Calculated cumulative paternity index = 219,077. Likelihood of paternity = 99.9995%.

spontaneous de novo occurrence of AT deficiency, in a 17-year-old male with a history of thrombophilia and a negative family history.

PATIENT AND METHODS

Patient History

A 17-year-old male presented with his first thromboembolic episode with extensive deep vein thrombosis (DVT) of the right leg at 12 years of age. The acute thrombus was treated with heparin, followed by warfarin. Because of persistent venous insufficiency, long-term warfarin therapy was prescribed. AT deficiency was diagnosed five months later. Results of the relevant diagnostic tests, done at the time of diagnosis of AT deficiency are shown in Table I. The AT activity by chromogenic assay, at that time, was 58% (normal range 84–123%).

Eleven months later he experienced a recurrence of the DVT in the right leg. This event was managed in a similar manner as the initial event, with the addition of AT concentrate replacement (50 IU/kg body weight/day) for one week. Five months later, during maintenance Coumadin® therapy, a thrombus of the left posterior inferior cerebellar artery was diagnosed; it was causing a persistent visual field deficit. This acute episode was again treated with a combination of AT concentrate and heparin, followed by Coumadin®. Prophylactic infusions of AT concentrate were given for one year following this event. One additional episode of right-leg DVT occurred despite prophylactic oral anticoagulant therapy. Two years following the cerebellar artery occlusion, and after discontinuation of the prophylactic AT infusions, the patient experienced acute chest pain and shortness of air. A pulmonary embolus was diagnosed. The patient received thrombolytic therapy, followed by AT replacement and intravenous anticoagulation with heparin. The patient is currently maintained with Coumadin® anticoagulation and twice-weekly AT prophylactic infusions (3,000 IU/dose). The propositus and his parents gave written consent for the clinical and molecular studies.

Methods

Clinical coagulation studies. These were performed by Colorado Coagulation Consultants, Inc. (Denver, CO), using frozen, citrated plasma samples. The laboratory values presented in Table I represent the results of various coagulation assays, performed at a time remote from any clinical thromboembolic event and during a temporary hiatus from anticoagulation therapy.

Genetic marker studies. The ten genetic marker studies, listed in Table II, were performed using genomic DNA extracted from whole blood mononuclear cells, from the propositus, both parents, and one male sibling. The methods used to detect the various polymorphisms were done according to the references given in Table II.

Antithrombin gene sequence. The sequence of the AT gene of the propositus was determined using polymerase chain reaction (PCR)-amplified DNA of each exon of the propositus. As the point mutation in the AT gene was present only in exon 5, sequencing of the AT gene of the family members was limited to exon 5. Oligonucleotide pairs were purchased from the Central Fa-

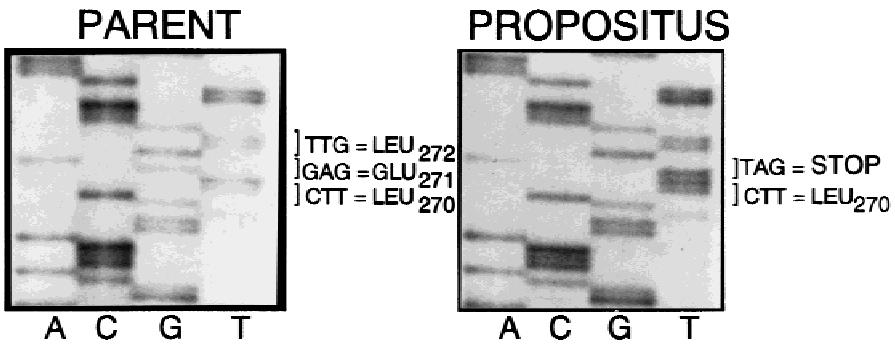

Fig. 1. Photograph of the sequence autoradiograph for the mutant AT allele of the propositus and that from one parent. The brackets mark the sequence of the various codons shown, as well as the corresponding amino acid. The STOP codon at position 271 is noted in the sequence of the propositus.

cility of the Institute for Molecular Biology and Biotechnology, McMaster University (Hamilton, Ontario). Sequence was obtained directly from PCR products using rapid cloning of PCR-amplified DNA. The oligonucleotide pair flanking exon 5 was: (sense) 5′ AUGGAGAUCUCUTGTTCTTAC-TTTGTGATTCT 3′, and (antisense) 5′ ACGCGUACUAGUAAACTCCTTC-CTAGACAAAC 3′. Multiple clones were sequenced for each allele to rule out potential sequencing artifacts, due to nucleotide misincorporation during PCR amplification.

RESULTS

Genetic marker studies. The results of the genetic marker studies for the propositus, a male sibling, and both parents are presented in Table III. For each marker, the paternity index [5–7], or odds in favor of paternity, is listed. From these results, the calculated cumulative paternity index (CPI) was determined to be 219,077, with the likelihood of paternity estimated to be 99.9995% [8,9].

Antithrombin gene sequence. The sequence radiographs for the mutant exon 5 allele of the propositus and the corresponding normal AT allele of one parent are shown in Figure 1. The nucleotide sequence numbering used is according to Olds et al. [10]. Codons 271 in the parents consists of GAG which codes for Glu. In the propositus codon 271 consists of TAG which codes for a STOP signal. Table IV shows the AT alleles for codon 271 in this family. Only the propositus is heterozygous for the mutant 271 TAG allele.

TABLE IV. Codon 271 Sequenced in Kindred

Identification	DNA sequence	Amino acid coded
Propositus	TAG/GAG	STOP/glu[a]
Mother	GAG/GAG	glu/glu
Father	GAG/GAG	glu/glu
Brother	GAG/GAG	glu/glu

[a]Glutamic acid.

DISCUSSION

Inherited AT deficiency is an autosomal dominantly inherited disorder with an estimated prevalence of 1:500 to 1:3000 in the general population [1,11,12]. At the time of publication of the AT mutation database 2nd update [1], 102 entries described kindreds with distinct type I AT deficiency and 127 described kindreds with distinct type II or ''variant'' AT deficiency. Several have been reported separately describing kindreds or families with novel AT mutations [13–17]. Those resulting in type I deficiency include a nonsense mutation Arg197STOP [13], a Gly424Arg amino acid substitution [14], a Pro80Thr substitution [15], and a His120Tyr substitution [15]. van Boven et al. [16] reported the molecular basis of AT deficiency in eight Dutch families and found point mutations leading to frameshift, premature termination, and amino acid substitution mutations. Daly et al. [17] reported five novel mutations resulting in type I AT deficiency in six unrelated kindreds. All produced premature termination codons. None of these are reported as de novo mutations. Recently reported mutations resulting in type II AT deficiency include Met251Ile [15] with decreased heparin affinity and Arg393His [18] with increased heparin affinity. Neither are the result of spontaneous mutations.

Unlike other inherited genetic disorders, spontaneous mutations accounting for AT deficiency appear to be rare occurrences. An AT gene mutation producing a type II pleiotropic defect, AT Torino, was identified in an Italian young man whose parents had normal antigenic and functional levels of AT [19]. The Torino allele (exon 6, codon 402) is also a single base substitution resulting in a phenylalanine to serine substitution. Although the details are not provided in the report, genetic linkage studies are reported to have supported the assigned genetic relationships among the various family members. To date, the AT-Torino allele represents the only report of a de novo mutation resulting in type II AT deficiency.

Reduced antigenic and functional levels of AT in our patient were the basis for the phenotypic diagnosis of type I AT deficiency. The relatively severe clinical course, at an early age, prompted the search for concomitant causes of thrombophilia in the propositus. The discovery of the novel codon 271 mutation in the proposi-

tus, and normal alleles (as well as normal antigenic and functional AT levels) in both parents, prompted linkage studies to verify the genetic relationships of various members of the kindred. The panel of markers chosen conferred an extremely high degree of certainty for conclusions regarding maternity and paternity for this AT-deficient patient, as shown in Table III. Thus, the absence of an AT gene mutation in the parents proves that this AT defect in our patient is the result of a spontaneous mutation. This is the first report of a de novo novel mutation in a thrombophilic individual with type I AT deficiency.

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